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New Aspect of Bone Morphogenetic Protein Signaling and Its Relationship with Wnt Signaling in Bone

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1. Introduction

Bone morphogenetic proteins (BMPs) were discovered and named in 1965 by Marshall Urist, who initially identified the ability of an unknown factor in bone to induce ectopic bones in muscle [1]. In the last 45 years, the osteogenic function of BMPs has been extensively examined, mainly using osteoblasts in culture with exogenous treatments of BMPs [2]. Based on their potent osteogenic abilities, clinical trials have been initiated to use BMP2 and BMP7 to improve fracture repair [2]. The FDA (Food and Drug Administration) has approved BMP2 and BMP7 for clinical use in long bone open-fractures, non-union fractures and spinal fusion. However, recent clinical/pre-clinical studies have shown a negative impact of BMPs on bone formation under certain physiological conditions [3-7], challenging the current dogma. This book chapter will focus on the recent findings of roles of BMP signaling in bone including its relationship with Wnt signaling through Wnt (Wngless, Int-1) receptor SOST (Sclerostin) and DKK1 (Dickkopf1). This new molecular interaction would explain the negative outcomes of BMP's therapy in orthopaedics.

2. Signaling by BMPs

Marshall Urist made the key discovery that demineralized bone matrix induced bone formation in 1965 [1]. It took another 24 years for BMPs to be discovered. The combined works of several researchers led to the isolation of BMPs and later the cloning [8-11]. BMPs belong to the transforming growth factor-β (TGF-β) gene superfamily [12]. Like other members of the TGF-β family, BMPs signal through transmembrane serine/threonine kinase receptors such as BMP type I and type II receptors. Upon ligand binding, type I and II receptors form hetero-multimers [13], and the type II receptor phosphorylates and activates a highly conserved glycine- and serine-rich domain (TTSGSGSG) called a GS box between the transmembrane and kinase domains in the type I receptor. The activated BMP type I receptors relay the signal to the cytoplasm through the Smad (Sma and Mad related protein) pathway by phosphorylating their immediate downstream targets, receptor-regulated Smads (R-Smads; Smad1, Smad5, and Smad8) proteins, which then interact with co-Smad (Smad4) protein and translocate into the nucleus [14]. It is also known that non-Smad

pathways through p38 MAPK (mitogen-actiated protein kinase) and TAK1 (Transforming growth factor β–activated kinase 1) are also involved in the BMP signaling [15]. There are three type I receptors [BMPRIA (BMP receptor type IA, ALK3), BMPRIB (BMP receptor type IB, ALK6) and ACVRI (Activin receptor type I, ALK2) and three type II receptors [BMPRII (BMP receptor type II), ACVRIIA (Activin receptor type IIA) and ACVRIIB (Activin receptor type IIB)], and approximately 30 ligands are identified [16]. Type I receptor ACVRI was originally described as an activin receptor, but it is now believed to be a receptor for BMPs. In osteoblasts, BMP2, BMP4, BMP6 and BMP7 and their receptors BMPRIA and ACVRI are abundantly expressed [17]. BMPRIA is a potent receptor of BMP2 and BMP4 [18, 19], as is ACVRI for BMP7 [20]. In addition, BMP antagonists Noggin, Chordin, and Gremlin were identified in osteoblasts [21]. These antagonists fine-tune BMP signaling in osteoblasts, as BMPs upregulate expression levels of antagonists while inducing BMP signaling [22] **(Table 1, Figure 1)**.

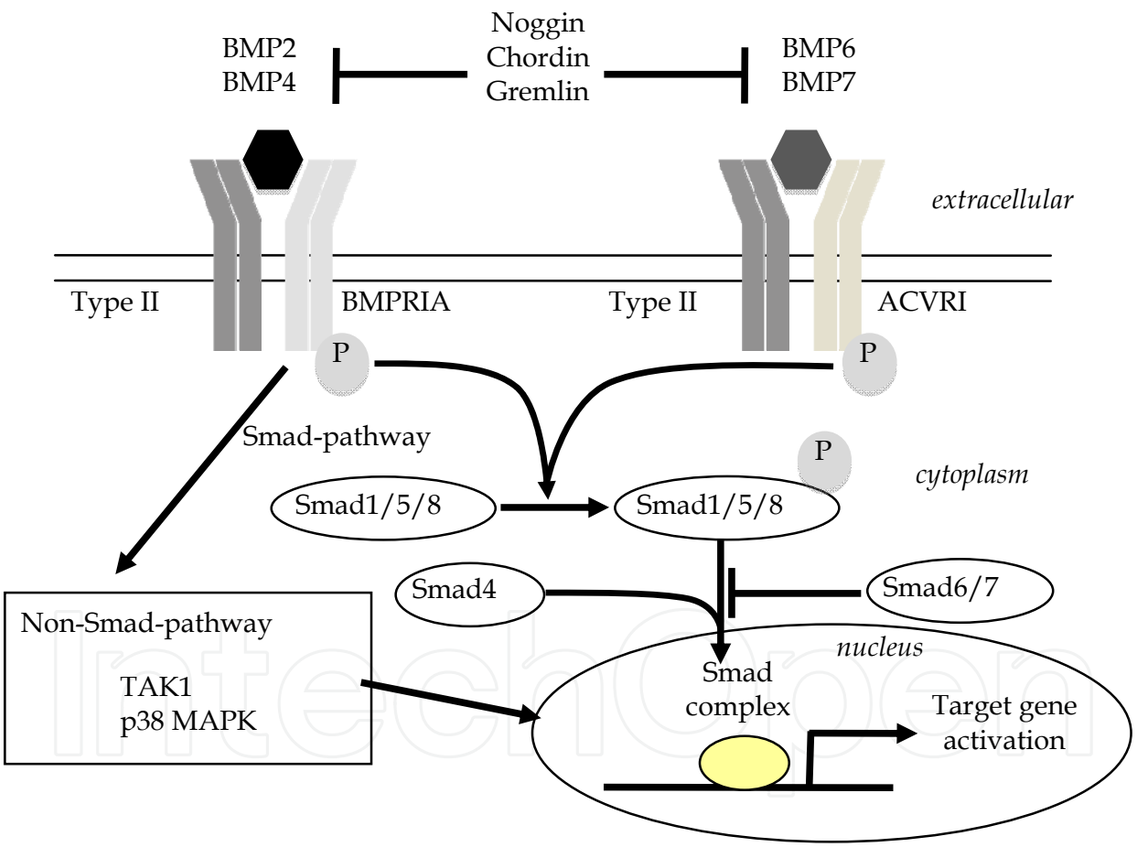

Fig. 1. Potential molecular interaction of BMP signaling in osteoblasts. BMP2, BMP4, BMP6, and BMP7 are osteoinductive and are expressed by osteoblasts. BMP2 and BMP4 are potent ligands for BMPRIA as are BMP6 and BMP7 for ACVRI. Canonical BMP signaling is through the Smad pathway via Smad1, Smad5, and Smad8 (i.e. Smad1/5/8-Smad4 complex), while non-canonical BMP signaling is through non-Smad pathways including TAK1 and p38 MAPK. Target genes are activated by these two pathways in osteoblasts.

Antagonists	Noggin, Chordin, Gremlin
Ligands	BMP2, BMP4, BMP6, BMP7
Type I Receptors	BMPRIA/ALK3, ACVRI/ALK2, (BMPRIB/ALK6)
Type II Receptors	BMPRII, ActRIIA, ActRIIB
R-Smad	Smad1, Smad5, Smad8
Co-Smad	Smad4
Non-Smad Pathways	p38 MAPK, TAK1

Table 1. Osteogenic BMPs and their signaling cascades in osteoblasts

3. Molecular interaction of BMP and Wnt

In addition to BMP signaling, Wnt signaling has been examined for a decade because of its role in bone formation and bone mass [23-27]. The physiological impact of Wnt signaling on bone mass was first reported in 2001, by showing that loss-of-function mutations in the co-receptor LRP5 (Low-density lipoprotein receptor-related protein 5) cause the autosomal recessive disorder osteoporosis-pseudoglioma syndrome (OPPG), a low bone mass phenotype in humans [28]. The importance of other Wnt ligands and receptors as bone mass effectors has been documented using genetic approaches for DKK1 [29], DKK2 [30], sFRPs (secreted frizzled-related proteins) [31], Sost/sclerostin [32], Lrp5 [33, 34] and Lrp6, all of which are expressed in osteoblasts. However, changes in BMP signaling in bone had not been reported in Wnt-related mutations in mice.

3.1 In vitro relationship

In vitro experiments using pluripotent mesenchymal cell lines or primary osteoblasts to test the interaction between BMP and Wnt signaling in osteoblasts have yielded both synergistic and antagonistic results: the treatment of C2C12 cells and primary osteoblasts with BMP2 induced Wnt3a expression and stabilized Wnt/β-catenin signaling [35-37]. The treatment of C3H10T1/2 cells with Wnt3a induced the BMP4 expression levels [38]. These suggest a positive autocrine loop [37, 39]. In contrast, inhibition of BMP signaling by treatment of primary osteoblasts with dorsomorphin, an inhibitor of BMP type I receptors, increased canonical Wnt signaling [40]. Treatment of C2C12 cells with Wnt3a repressed BMP2-dependent *Id1* (Inhibitor of DNA binding 1) expression [41]. Similarly, treatment of cultured skull bone with BMP antagonist Noggin increased canonical Wnt signaling [42]. Moreover, one study investigated intracellular cross-talk between BMP and Wnt pathways using uncommitted bone marrow stromal cells and provided a potential mechanism whereby BMP-2 antagonizes Wnt3a-induced proliferation in osteoblast progenitors by promoting an interaction between Smad1 and Dvl-1 [i.e. the human homolog of the Drosophila dishevelled gene (dsh) 1] that restricts Wnt/β-catenin activation[43]. Another interaction via Pten (phosphatase and tensin homolog)-Akt pathway has been reported in hair follicle stem/progenitor cells [44]; however, it is less likely in osteoblasts [45]. Taken together, there seems to be both positive and negative feedback loops between the two signaling pathways **(Figure 2)**.

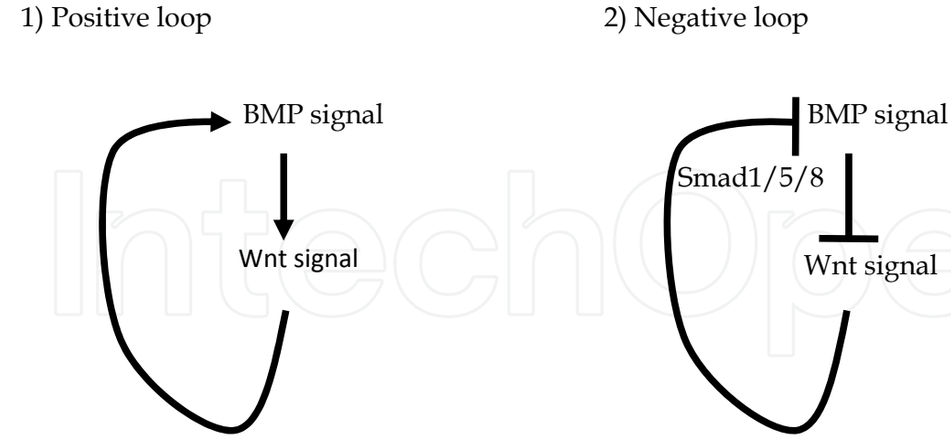

Fig. 2. A potential relationship between the two major signaling BMP and Wnt in osteoblasts based on in vitro studies. 1) Both signaling pathways function in a positive loop. 2) Both signaling pathways function in a negative loop. It is expected that these two signaling pathways may regulate each other in an age-dependent and context-dependent manner. Further studies are desired to investigate the details of each condition.

3.2 In vivo relationship

In vivo, only a few studies have revealed a link between the two signaling pathways. We recently found that loss-of-function of BMP signaling in osteoblasts via BMPRIA upregulates canonical Wnt signaling during embryonic and postnatal bone development, suggesting a negative regulation of Wnt signaling by BMP [40, 42]. In these studies, we found that upregulation of Wnt signaling is at least in part mediated by suppression of Wnt inhibitors Sost/sclerostin and Dkk1, and both Sost/sclerostin and Dkk1 are direct targets of BMP signaling. In addition, *Sost* expression was severely downregulated in *Bmpr1a*-deficient bones as assessed by microarray analysis [42]. Interestingly, both Smad-dependent and Smad-independent pathways appear to contribute to the Dkk1 expression, whereas Sost/sclerostin requires only Smad-dependent signaling, suggesting differential regulation of these genes by the BMP signaling via BMPRIA [40]. BMP and Wnt signaling regulate the development and remodeling of many tissues and interact synergistically or antagonistically in a context- and age-dependent manner *in vivo* [46, 47]. It is possible that in bone, BMP signaling inhibits Wnt signaling by upregulating the Sost/sclerostin expression in osteoblasts **(Figure 3)**.

3.3 SOST/Sclerostin and DKK1

Both SOST and DKK1 are inhibitors for canonical Wnt signaling and have been highlighted because neutralizing antibodies for SOST (AMG785) and DKK1 (BHQ880) have been developed as bone anabolic agents and these potential drugs are under clinical trial [48]. It is known that both Dkk1 and Sost/sclerostin inhibit Wnt/β-catenin signaling by binding to co-receptors. As both Dkk1 and Sost/sclerostin are secreted proteins expressed by osteoblasts, their role in regulating bone mass has been investigated using human and mouse genetic approaches.

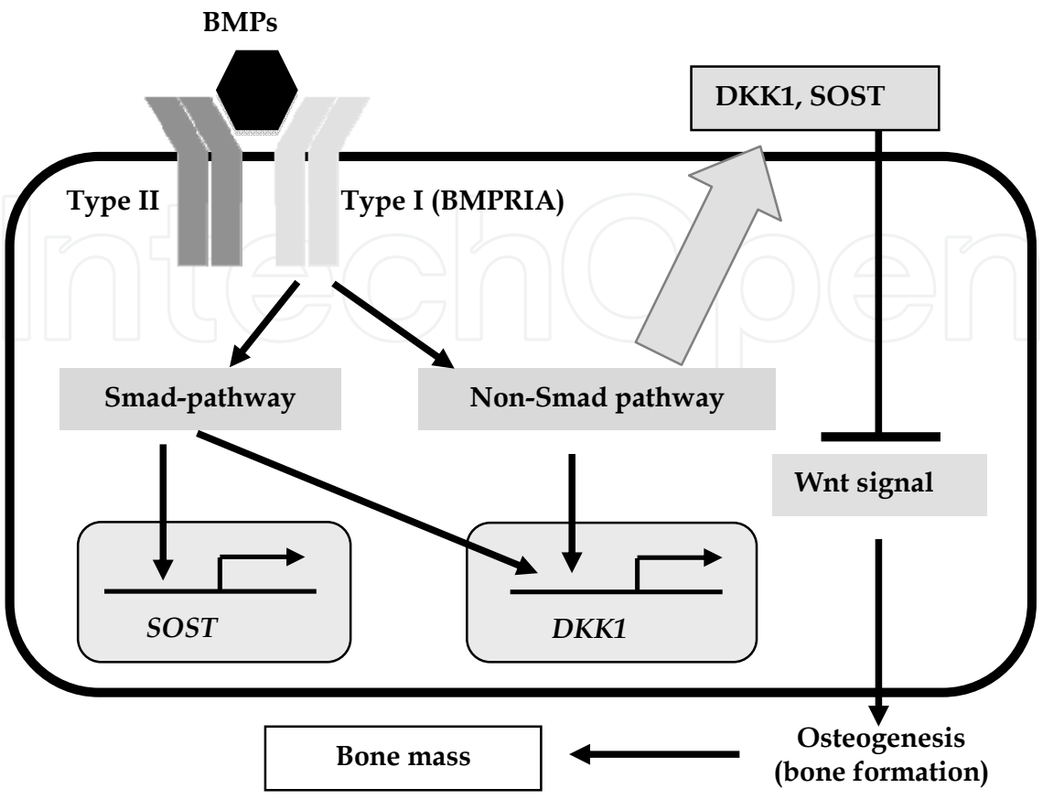

Fig. 3. Possible regulation between BMP and Wnt in Osteoblasts. A proposed model of the relationship between the BMP signaling via BMPRIA and the canonical Wnt signaling in osteoblasts. Both Dkk1 and sclerostin/Sost are downstream targets of the BMP signaling. The BMP signaling upregulates the Sost expression primarily through the Smad-dependent signaling while it upregulates the Dkk1 expression through both the Smad and non-Smad signaling (p38 MAPK). As Dkk1 and sclerostin/Sost act as Wnt signaling inhibitors, BMP signaling in osteoblasts, in turn, leads to a decrease in osteogenesis and bone mass. Dkk1 and sclerostin/Sost play an important role in regulating bone mass as downstream effectors of BMPRIA signaling in bone taking balances between BMP signaling and Wnt signaling.

3.3.1 SOST/Sclerostin

Sost/sclerostin was originally reported as a member of the BMP antagonist DAN family (i.e. the Dan gene family of BMP antagonists) [49, 50]. Although DAN family members modulate both BMP and Wnt signaling in Xenopus [51-53], recent studies suggest a primary role of Sost/sclerostin in Wnt signaling in mouse and humans: Sost/sclerostin is not a BMP antagonist [54] but rather a Wnt inhibitor [55] that binds the Wnt co-receptor low density lipoprotein receptor-related protein 5 and 6 (LRP5 and LRP6) [32, 56]. Conventional knockouts of *Sost* (i.e. *Sost* KO) are viable and exhibit increased bone mass [57]. In humans, loss-of-function and hypomorphic mutations in *SOST* cause sclerosteosis [58, 59] and Van Buchem disease [60, 61], respectively, with a high bone mass (HBM) phenotype. These mutants share the HBM phenotype with other gain-of-function of LRP5 mutation, due to the defect in DKK1-mediated regulation of LRP5 in humans [62-64] and overexpression of Lrp5 in mice [65]. In

contrast, loss-of-function of LRP5 leads to OPPG with low bone mass [28], which is similar to the bone phenotype of mice overexpressing *Sost* [50]. In addition, recent genome-wide SNP-based analyses identified a significant association between bone mineral density and the *SOST* gene locus [66-68].

3.3.2 DKK1

Conventional knockouts of *Dkk1* die *in utero* from defective head induction and limb formation [29]. Similar to *Sost* KO mice, mice heterozygous for *Dkk1* (*Dkk1*$^{+/-}$ mice), however, exhibit a high bone mass (HBM) phenotype [69], while overexpression of *Dkk1* in osteoblasts causes osteopenia [70]. In addition, increased *DKK1* expression in bone marrow has also been associated with lytic bone lesions in patients with multiple myeloma [71]. Collectively, these results support the hypothesis that Dkk1 functions as a potent negative regulator of bone mass.

3.3.3 Sost/DKK1 expression in the *Bmpr1a* cKO mice

Conditional knockouts of *Bmpr1a*, which are deficient in the *Dkk1* and *Sost* expression, show a HBM phenotype [40, 42, 72]. In particular, Sost expression levels were the most dramatically reduced in the cKO mice during embryonic stages [42]. Furthermore, both Sost and Dkk1 expression levels were increased by the addition of BMP2, a potent ligand for BMPRIA, using primary osteoblasts [40]. Similarly, both Sost and Dkk1 expression levels were significantly reduced in the *Acvr1* cKO mice [73]. In addition, both Sost and Dkk1 expression levels were increased by the addition of BMP7, a potent ligand for ACVRI, using primary osteoblasts [73]. These facts support the new concept of molecular interactions between BMP signaling and Wnt signaling that Dkk1 and Sost/sclerostin act physiologically as inhibitors of canonical Wnt signaling as downstream targets of BMP receptors BMPRIA and ACVRI and that BMP signaling can negatively controls Wnt signaling in osteoblasts **(Figure 3)**.

3.4 Effects of Wnt signaling on osteoclasts

There is accumulating evidence that Wnt signaling also plays a critical role in osteoclastogenesis regulated by osteoblasts through the RANKL (Receptor activator of nuclear factor kappa-B ligand)-OPG (Osteoprotegerin) pathway. Recently, two *in vivo* studies have suggested that the canonical Wnt signaling is important in the regulation of osteoclastogenesis by osteoblasts. One study provided evidence that the Wnt pathway positively regulates the expression of *Opg* in osteoblasts [74]. Overexpression of stabilized β-catenin in osteoblasts, which results in an increase of canonical Wnt signaling level, decreases osteoclast differentiation leading to increased bone volume in mice [74]. Another study showed that an osteoblast-specific deletion of β-catenin leads to an impaired maturation and mineralization of bones in mice due to the elevated expression of *Rankl* and diminished *Opg* [75]. These facts suggest that the canonical Wnt pathway negatively regulates osteoblasts in their supporting function in osteoclastogenesis, and thus upregulation of Wnt signaling in osteoblasts can suppress osteoclast-mediated bone resorption [75]. Taken together, it is possible that the treatment of bones with BMPs can reduce Wnt activity in osteoblasts and in turn enhance osteoclast activity.

4. BMP signaling and mouse genetics

Along with the huge advancement in technologies involving mouse genetics over the last decade, many of the BMP signaling related genes have been knocked out in mice. BMP2, BMP4, BMP6 and BMP7 and their receptor BMPRIA and ACVRI are abundantly expressed in bone. However, conventional knockout mice for these genes result in an early embryonic lethality and thus, it is not possible to investigate bone development and remodeling using these models [76-82]. To avoid the embryonic lethality, a strategy of conditional knockout mice using a Cre-loxP system has been employed. A bone-specific conditional deletion of *Bmpr1a* using an *Og2-Cre* mouse, in which a Cre recombination is restricted in differentiated osteoblasts under the osteocalcin promoter, was first reported in 2004 [83]. Interestingly, this study demonstrated that the response of osteoblasts to BMP signaling is age-dependent; in the mutant mice, bone volume decreased in young mice but increased in aged mice. In addition, the activity of osteoclasts was reduced in the aged osteoblast-specific *Bmpr1a*-deficient mice, which may have lead to the complex skeletal phenotype. These facts suggest that the BMP signaling in differentiated osteoblasts can control the balance between bone formation by osteoblasts and resorption by osteoclasts, thereby affecting the final outcome of the amount of bone mass in an age-dependent manner. The increased bone mass in the *Bmpr1a*-deficient mice appeared to be in opposition to the general concept of BMPs as osteogenic inducers; however, the concept is reasonable if the target cell for BMPs as osteogenic inducers is mesenchymal cells or chondrocytes,. It is expected that BMPs have multifaceted functions *in vivo* because different cell types exhibit differing responses to BMPs. In addition, the opposite outcome in the *Bmpr1a*-deficient mice was discussed from the point of molecular interaction in the sections 3.

4.1 BMP signaling in chondrocytes, mesenchymal cells, and osteoblasts

During skeletogenesis, bones are formed via two distinct processes: intramembranous and endochondral bone formation [84]. Intramembranous bone formation occurs primarily in flat bones (*e.g.*, calvarial bones) where mesenchymal cells differentiate directly into osteoblasts [85]. Endochondral bone formation occurs primarily in long bones where condensed mesenchymal cells differentiate into chondrocytes to form cartilage templates, and then chondrocytes are replaced by osteoblasts [86]. Recently many studies have been designed to investigate the difference in the molecular mechanism by which BMP signaling regulates these cell types. Several Cre mouse lines have been used to target different cell types including osteoblast, chondrocyte, and mesenchymal cells **(Table 2).** BMP signaling in chondrocytes and mesenchymal cells both positively control bone size and mass while BMP signaling in osteoblasts can reduce them.

4.1.1 Chondrocytes

There are several lines of evidence that show that BMP signaling in chondrocytes is required for bone size and the amount of bone mass. BMP signaling through BMPRIA is essential for postnatal maintenance of articular cartilage, using a *Gdf5*-Cre mouse line specific for chondrocytes in joints [87]. Similarly, the critical role of *Bmpr1a* together with *Bmpr1b* in chondrocytes during endochondral bone formation using a *Col2*-Cre mouse line was reported.[88]. Moreover, in chondrocytes a simultaneous deficiency in Smad 1 and Smad 5,

	Promoter Cre-mouse	*BMP signal*	*Stage*	*Bone mass*	*Ref.*
Chondrocyte					
Bmpr1a cKO	Gdf5-Cre	down	E12.5-E16.5, 7W, 9	Reduced	87
Double knockout of *Bmpr1a* and *Bmpr1b*	Col2-Cre	down	E12.5-E16.5	Reduced	88
Bmp4 overexpression	Col11a2	up	E18.5	Increased	89
Noggin overexpression	Col11a2	down	E18.5	Reduced	89
Double knockout of *Smad1* and *Smad5*	Col2-Cre	down	E12.5-newborn	Reduced	90
Mesenchymal cell					
Double knockout of B_{MP2} and B_{MP4}	Prx1-Cre	down	E10.5-newborn, 3W	Reduced	91
Bmp2 cKO	Prx1-Cre	down	5M	Reduced	92
Osteoblast					
Bmpr1a cKO	Ogl2-Cre	down	3M 10M	Reduced Increased	83
Bmp4 overexpression	2.3 kb Col1	up	E18.5	Reduced	93
Noggin overexpression	2.3 kb Col1	down	E17.5, 3W	Increased	93
Bmpr1a cKO	3.2 kb Col1-CreER	down	E18.5, 3W, 5M	Increased	40, 42, 72
Acvr1 cKO	3.2 kb Col1-CreER	down	E18.5, 3W, 5M	Increased	73
Osteoclast					
Bmpr1a cKO	Ctsk-Cre	down	8W	Increased	94

Table 2. Bone mass observed in genetically engineered mutant mice of BMP signaling

which are BMPs' downstream target molecules, reduces bone mass [90]. In parallel, studies focusing on BMP ligands and their antagonists provide further evidence that BMPs are critical for normal development of cartilage. A transgenic mouse line to overexpress *Bmp4* in mesenchymal cells/chondrocytes using a type XI collagen promoter (Col11a2) was generated, and bone mass was increased in the mutant mice [89]. Another transgenic mouse line in which *Noggin* was overexpressed in the same cells (*Col11a2-Noggin*) demonstrated a decreased bone mass. As Noggin is an antagonist for BMPs (BMP2, BMP4, BMP5, BMP6, and BMP7) with various degrees of affinity [95], these results suggest that BMP signaling positively controls proliferation and differentiation of chondrocytes.

4.1.2 Mesenchymal cells

Similar to chondrocytes, a few studies demonstrated a requirement of BMP signaling in mesenchymal cells for proper bone development and remodeling using a mesenchymal cell-specific Cre mouse line, *Prx1-Cre*, in which Cre is active in mesenchymal cells as early as embryonic day 9.5 [96]. Using the *Prx1-Cre* mouse, the simultaneously conditional deletions of *Bmp2* and *Bmp4* in mesenchymal cells resulted in an impairment of osteogenesis during late embryogenesis [91, 92]. In contrast, the conditional deletion of *Bmp2* in mesenchymal cells does not show overt developmental abnormalities; however, the resulted mice lack an initiation of fracture healing [91, 92]. Interestingly, *Bmp7*-deficiency in mesenchymal cells did not affect bone mass probably due to the compensation by Bmp4 [97]. Taken together, it is possible that the defects in the BMP signaling in chondrocytes largely contribute to the phenotypes described above because chondrocytes are derived from mesenchymal cells and play an important role in the process of fracture repair.

4.1.3 Osteoblasts

As aforementioned, a differentiated osteoblast-specific deletion of *Bmpr1a* caused an increase in bone mass in aged mice [83]. Similar to this finding, an overexpression of a BMP antagonist, Noggin, in osteoblasts increases bone volume with a reduced osteoclast number and osteoclastogenesis both at embryonic day 17.5 (E17.5) and at 3 weeks [93]. In parallel, the overexpression of *Bmp4* in osteoblasts reduced bone mass presumably due to the increase in the osteoclast number at E18.5 [93]. Recently, *Bmpr1a* was conditionally disrupted in immature osteoblasts using a tamoxifen inducible Cre driven by a 3.2-kb alpha1(I) collagen chain gene (Col1a1) promoter. In the mutant mice, bone mass was dramatically increased during the

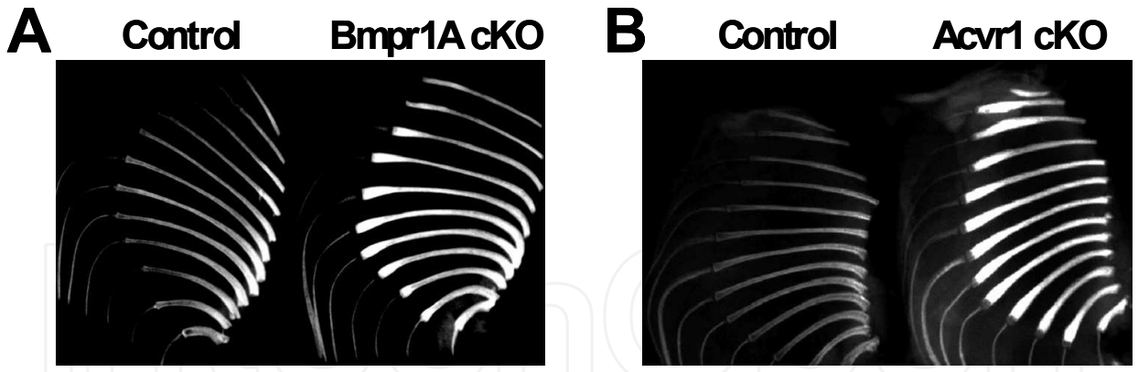

Fig. 4. Increased bone mass in the osteoblast-specific conditional knockout (cKO) mice for BMP receptors BMPRIA or ACVRI at the adult stage. *Bmpr1a* or *Acvr1* cKO mice were generated by crossing a floxed mouse line for *Bmpr1a*(*Bmpr1a*fx/fx) or *Acvr1*(*Acvr1*fx/fx) with a transgenic mouse line harboring a tamoxifen–inducible Cre driven by a 3.2 kb mouse procollagen α1(I) promoter. The Cre recombination was induced specifically in the osteoblasts by 10 weeks of tamoxifen administration from 10 weeks after birth, and bones were removed at 22 weeks. Radiodensity of rib bones was assessed by X-ray. (A) The radiodensity was dramatically increased in the *Bmpr1a* cKO mice (Cre+, *Bmpr1a*fx/fx) compared with controls (Cre–, *Bmpr1a*fx/fx). (B) The radiodensity was dramatically increased in the *Acvr1* cKO mice (Cre+, *Acvr1*fx/fx) compared with controls (Cre–, *Bmpr1a*fx/fx).

bone remodeling stage at 22 weeks as well as the bone developmental stages at E18.5 and 3 weeks [42, 72] **(Figure 4A).** This result is an interesting contrast to previous work that disruption of *Bmpr1a* in differentiated osteoblasts results in decrease of bone mass in young adult stages (3-4 weeks). The increased bone mass in the *Bmpr1a*-deficient mice resulted from severely suppressed bone resorption due to reduced osteoclastogenesis, despite a simultaneous small reduction in the rate of bone formation [72]. Levels of RANKL and OPG are changed in the *Bmpr1a*-deficient osteoblasts and fail to support osteoclastogenesis [42, 72]. In addition, the conditional disruption of *Acvr1* in osteoblasts also demonstrated a dramatic increase in bone mass, similar to the bone phenotype of *Bmpr1a*-deficient mice **(Figure 4B)**, although osteoclastic activity is still under investigation [73]. These findings suggest that BMP signaling may have dual roles in osteoblasts; to stimulate both bone formation by osteoblasts and bone resorption supporting osteoclastogenesis. Disruption of BMP signaling in immature osteoblasts alters the balance of bone turn over to increase the bone mass, which is opposite to what people have expected for the past 4 decades.

4.1.4 Other cell type

Angiogenesis is another necessary step in new bone formation in skeletal development as well as in bone remodeling after fracture [98, 99]. Both BMP2 and BMP7 are known to induce angiogenesis by associating with other growth factors such as VEGF (vascular endothelial growth factor), bFGF (basic fibroblast growth factor), and TGF-β1 [100]. A study using an adenovirus vector in muscle demonstrated that BMP9 induces ectopic bone formation similar to BMP2 [101, 102]. As BMP9 is abundantly expressed in endothelial cells that are primarily cell types for angiogenesis [103], it is possible that BMP signaling in endothelial cells synergizes anabolic bone formation. The mechanism and origin of precursor cells for ectopic bone formation, which is physiologically observed in the patients with FOP (fibrodysplasia ossificans progressiva), is under investigation [104-106] but could be endothelial cells [107].

4.1.5 Possible interpretation

Mesenchymal cells, chondrocytes, and endothelial cells respond to BMPs by inducing bone mass and size **(Table 3)**. Recent histological findings suggest that the process of endochondral bone formation, which first forms cartilage template prior to the final bone following vessel formation (i.e. angiogenesis), plays a critical role in the process of ectopic bone formation [108]. The origin of precursor cells for the ectopic bone is under investigation [105, 106]; however, it is possible that formation of ectopic bones by BMPs [1] is largely due to the stimulation of chondrocytes, mesenchymal cells, and/or endothelial cells in soft tissue, which results in an expansion of ectopic cartilage subsequently replaced by osteoblasts. There is another possibility that the BMP signaling directly affects osteoblasts to form ectopic bone. However, this possibility is less likely based on recent evidence that reduced

Cell types that can increase bone mass	Cell types that can reduce bone mass
Mesenchymal cells Chondrocytes Osteoblasts Endothelial cells	Osteoclasts Osteoblasts

Table 3. A variety of cell types in bone that mediate bone mass in response to BMPs

BMP signaling in osteoblasts results in an increase in bone mass. As current methods of systemic and local treatment affect multiple cell types simultaneously in bone, it is important to evaluate the effects of BMPs on more than just osteoblasts.

4.2 Effect of BMP signaling on osteoclasts

Bone mass is determined by the balance between bone formation and bone resorption. Osteoclasts are multinuclear cells derived from hematopoietic stem cells to secrete enzymes for bone resorption [109]. Recent mouse genetic studies revealed the importance of BMP signaling for osteoclastic activity and bone resorption.

4.2.1 Regulation of osteoclast by osteoblast-dependent BMP signaling

It is expected that BMPs play roles in osteoclastogenesis and their functions, because receptors for BMPs are expressed in these cells [110]. Additionally, osteoblasts also play critical roles in bone resorption by regulating osteoclastogenesis because they produce RANK ligand (RANKL), essential to promote osteoclastogenesis, and its decoy receptor, osteoprotegerin (OPG) [111, 112]. A balance between RANKL and OPG is important to determine the degree of osteoclastogenesis, i.e. more RANKL production by osteoblasts leads to more osteoclasts; thus more bone resorption is expected. As RANKL is an osteoblastic product and BMPs induce osteoblast maturation, BMPs indirectly stimulate osteoclastogenesis and thus, osteoclastogenesis is impaired when osteoblastogenesis is blocked with BMP antagonists in culture [113]. The physiological effects of BMP signaling in osteoblasts on osteoclastogenesis were determined later using an osteoblast-specific gain-of-function or loss-of-function mouse model. For the cases of the osteoblast-specific deletion of *Bmpr1a* and osteoblast-specific over expression of *Noggin*, osteoclastogenesis is highly compromised leading to an increase of bone mass [83, 93]. In contrast, osteoblast-specific overexpression of *Bmp4* increased osteoclastogenesis [93]. The regulation of RANKL by BMPs was suggested based on an *in vitro* study [114]. This concept was recently proven in mouse studies, as *Bmpr1a*-deficient osteoblasts were not able to support osteoclastogenesis due to an imbalance between RANKL and OPG [42, 72]. It is therefore concluded that osteoblasts can respond to BMPs by inducing osteogenic (i.e. bone anabolic) action as well as osteoclastogenic (i.e. bone catabolic) action simultaneously presumably dependening on context and timing **(Table 3).**

4.2.2 Regulation of osteoclast by osteoclast-dependent BMP signaling

BMP receptors are expressed in osteoclasts [110]. When BMP signaling through BMPRIA was deficient in osteoclasts using a Catepsin K promoter (CtsK), bone mass was increased as expected [94](Table 2). Interestingly, both bone formation rate and osteoblast number assessed by bone histomorphometry analysis were increased while osteoclast number was reduced in the mutant mice compared to their controls. It is possible that some coupling factors can control osteoblast function in an osteoclast-dependent manner in the mutant mice (i.e. osteoclast-derived coupling factors). Further studies are needed to determine whether such factors mediate BMPRIA-induced coupling from osteoclasts to osteoblasts.

5. Future direction of BMPs and Wnt

As is discussed in the former part of this review, it is important to understand that BMPs have variable and context-sensitive effects on diverse cell types in bone including

chondrocytes, osteoblasts, and osteoclasts. Studies focusing on BMP receptors in chondrocytes including mesenchymal cells suggest that these cells can respond to BMP signaling by increasing bone mass during the endochondral formation process. As discussed in the latter part, BMP signals can consistently inhibit Wnt signaling and bone mass while exerting concordant effects on *Dkk1* and *Sost*. This revision of traditional understanding of the BMP signaling pathway in clinical therapeutics might suggest that in some circumstances, BMP inhibition would be desirable for promoting bone mass. More importantly, if BMP signaling reduces bone mass by inhibiting Wnt signaling through SOST/DKK1 in osteoblasts, small molecule antagonists for BMPs or BMP receptors can conversely increase bone mass and size. Therefore, development of these molecules would be a next step towards disease conditions in which bone mass is reduced such as osteoporosis and bone fracture. Although antibodies for SOST and DKk1 have been developed in order to increase bone mass, the small molecule antagonists which can be an upstream of SOST and DKK1 would be used as more potent therapeutic agents for osteoporosis. Last, the function of the BMP signaling in osteoclasts remains largely unknown in terms of coupling factors and merits future study, although the BMP signaling regulates osteoblast-dependent osteoclastogenesis via the RANKL-OPG pathway.

6. Conclusion

Understanding the complex roles of the BMP signaling pathway and its molecular interaction with other signaling pathway (i.e. Wnt) in a variety of cell-types in bone including chondrocytes, osteoblasts and osteoclasts, which contribute to normal physiological conditions (i.e. bone development, homeostasis, and remodeling) will not only help to improve current knowledge of the pathological conditions (i.e. bone fracture, osteoporosis, and other congenital and aging-related bone diseases) but may provide novel therapeutically useful strategies.

7. Acknowledgment

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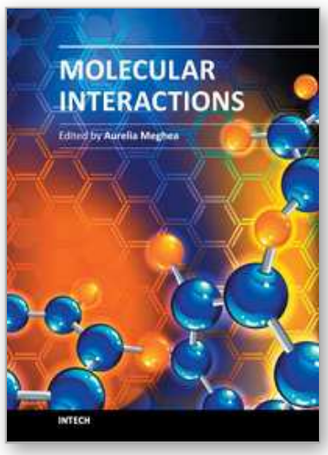

Molecular Interactions

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In a classical approach materials science is mainly dealing with interatomic interactions within molecules, without paying much interest on weak intermolecular interactions. However, the variety of structures actually is the result of weak ordering because of noncovalent interactions. Indeed, for self-assembly to be possible in soft materials, it is evident that forces between molecules must be much weaker than covalent bonds between the atoms of a molecule. The weak intermolecular interactions responsible for molecular ordering in soft materials include hydrogen bonds, coordination bonds in ligands and complexes, ionic and dipolar interactions, van der Waals forces, and hydrophobic interactions. Recent evolutions in nanosciences and nanotechnologies provide strong arguments to support the opportunity and importance of the topics approached in this book, the fundamental and applicative aspects related to molecular interactions being of large interest in both research and innovative environments. We expect this book to have a strong impact at various education and research training levels, for young and experienced researchers from both academia and industry.

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